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ORIGINAL PAPER

Combined osteomorphological, isotopic, aDNA, and ZooMS analyses of sheep and goat remains from Neolithic Ulucak, Turkey

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Abstract

The site of Ulucak is pivotal in exploring the Neolithic of the eastern Aegean and western Anatolia. It has an impressive stratigraphic sequence stretching from the first half of the seventh millennium BC to the first half of the sixth millennium BC. Recent zooarchaeological analyses have provided insight into the importance of animal husbandry practices and dairying at the site throughout the Neolithic but also raised questions about the changing nature of herd management strategies and whether these differed for sheep and goat. The faunal data, coupled with the significance of the site and condition of the assemblage, prompted the application of a number of methodological techniques to assess differences between sheep and goat. In this paper, we combine traditional osteomorphological analysis, ancient DNA, collagen peptide mass fingerprinting (zooarchaeology by mass spectrometry, or ZooMS), and stable isotope analysis of $\delta^{18}O$ and $\delta^{13}C$ from tooth enamel carbonate as well as $\delta^{13}C$ and $\delta^{15}N$ from bone collagen. As such, this is the first study of its kind. We evaluate the juxtaposition of these four approaches and their application in this important case, with relevance for future studies in the region.

Keywords Neolithic · Aegean · Stable isotopes · aDNA · Zooarchaeology · ZooMS

Introduction

Among the many advances in scientific archaeology in the last two decades, the addition of biomolecular and biogeochemical techniques to traditional osteological zooarchaeological analyses have contributed to solving questions regarding taxonomic affiliation using protein-based methods (e.g., Buckley et al. 2008, 2009, 2010), investigating phylogenetic relationships and demographic processes related to domestication using ancient DNA analysis (see Scheu 2017 for summary), and a more detailed consideration of ancient animal mobility and husbandry using stable isotope data (e.g., Pilaar Birch 2013; Makarewicz and Sealy 2015; Zangrando et al. 2014). Together with studies that review faunal data and the growth and development of open access databases (i.e., Arbuckle et al. 2014; Atici et al. 2013; Atici et al. 2017; Kansa et al. 2011; Orton et al. 2016), projects that combine multiple analytical approaches are becoming more commonplace. As this wealth of data increases, syntheses and integrative research grow ever more important in answering archeological questions.

Characterizing husbandry practices throughout the Neolithic, of which we know mainly from traditional zooarchaeology in Western Anatolia (Galik and Horejs 2011; Çakırlar 2012a), is critically important as agricultural lifestyles become established here during this time (Çilingiroglu and Çakirlar 2013; Özdoğan 2011). It has focused on first and foremost an assessment of the presence, absence, and relative abundance of domesticates and their relation to adjacent areas (Arbuckle et al. 2014; Conolly et al. 2011; Geörg 2013; Scheu et al. 2015; Ottoni et al.

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2013). At the multi-period site of Ulucak in western Turkey, previous zooarchaeological research has shown that sheep and goat herding was of primary importance and that there is a change in the relative abundances of sheep, goat, pig, and cattle remains throughout the Neolithic, indicative of decreasing economic importance of sheep and goat as proportions of pig and then cattle increase (Çakırlar 2012a, b). Analysis of mortality profiles and size-based reconstruction of sex discrimination at kill-off indicated that more specialized techniques were adopted in managing sheep and goat to meet the requirements for changes in targeted products (meat, milk, wool, herd size) throughout the Neolithic in the region (Arbuckle and Atici 2013; Çakırlar 2012a, b).

While these studies provide a firm foundation for studying animal husbandry in Neolithic Western Anatolia, they are limited in their power to address the nuances of sheep and goat herding (Çakırlar 2012a, b). The osteological distinction between sheep and goat is flawed (see below), despite the importance of ascertaining relative proportions of these animals in order to discern herding and slaughtering practices which may have distinguished quite starkly between the two; for example, herding goats for meat and sheep for wool. Likewise, differentiation of sex, often impossible based on morphology and only sometimes possible using measurements of fully fused skeletal elements, is critical for creating sex-specific age curves (Zeder 2001). Traditional ancient DNA (aDNA) analysis using targeted amplification and sequencing of short DNA fragments already has the potential to inform on taxonomic affiliation and identification of sex (e.g., Bar-Gal et al. 2003; Scheu et al. 2008) but is subject to high failure rates due to post-mortem DNA degradation, which is particularly true for our study region (Bollongino and Vigne 2008; Bollongino et al. 2008; Geörg 2013; Scheu et al. 2015). Collagen peptide mass "fingerprinting," the most popular form of ZooMS (Zooarchaeology by Mass Spectrometry; Buckley et al. 2009), is an effective way of discriminating between the main faunal species in this region (Buckley et al. 2010; Buckley and Kansa 2011), particularly the separation of sheep and goat (often grouped as ovicaprines), with much greater success rates in warmer sites dating to this period; however, it is not useful for sex determination. Finally, stable isotope analysis has the potential, using $\delta^{18}O$ and $\delta^{13}C$ from tooth enamel as tracers of climate and environment and $\delta^{13}C$ and $\delta^{15}N$ from bone collagen as indicators of diet, to inform on possible herding ranges, seasonal mobility, diet variability, birth season, and whether these were conservative through time (e.g., Balasse 2002; Balasse 2013; Pilaar Birch et al. 2016; Julien et al. 2012; Henton et al. 2010).

With continued focus on Ulucak, the type site for the Western Anatolian Neolithic, we used a combination of morphological criteria, aDNA, and ZooMS to resolve the taxonomic status of a subset of specimens prior to carrying out stable isotope analysis. We questioned how conservative herding practices for sheep and goat may have been through time and whether there was evidence that sheep and goat were managed differently, in terms of birth season, diet, and extent of seasonal mobility or range. Critically, assessing the integration of these multiproxy methods in our study design was as important a research goal as teasing out the nuances of Neolithic herding practices at Ulucak, and the study presented below focuses not only on the archeological aspects of the work but the multiple techniques used as well.

The site and its zooarchaeology

The site of Ulucak is located in the province of Izmir in western Turkey (Fig. 1). Situated on a fertile, well-watered inland plain at an elevation of about 215 m, it is approximately 9 km from the present-day Aegean coast (Çilingiroğlu and Abay 2005: 6). During the Neolithic, the coastline was no longer than a day's walk away (Çîlîngîroğlu et al. 2004), a walk that would have been made more difficult by a mountain pass that rises less than a kilometer west of the site (Çakırlar 2015). Ulucak is a typical multi-layered höyük, or tell site, with several architectural layers and occasional mixed deposits. It covers an area of 120×140 m^2 (visible at the surface of the plain) and rises about 8 m in height (Çevik 2013). Evidence for occupation spans from the Aceramic Neolithic to the Byzantine period (Çilingiroğlu et al. 2012; Çevik 2013). The generalized phases of the Neolithic occupation are numbered from VI to IV and range from between ca. 6700 to 5700 cal. BC (Table 1) (Çilingiroğlu et al. 2012). In Greece, Ulucak VI is roughly contemporary with the Late Mesolithic-Initial Neolithic Franchthi and Early Neolithic Knossos; Ulucak V is roughly contemporary with Early Neolithic Achilleon, Sesklo, and Nea Nikomedia on the mainland; and Ulucak IV corresponds with Middle Neolithic Achilleon and Franchthi, among others (c.f. www.14sea.org for individual references and Brami 2014). In Anatolia, Ulucak VI is contemporary with the earliest Çukuriçi and Barçın Höyük, and early Çatal East (but later than Aşıklı, the earliest Çatal East, and Mesolithic Girmeler); in the Turkish Aegean, it corresponds to the earliest evidence for occupation at the site of Uğurlu (level VI) on Gökçeada (Erdoğu 2016). Ulucak V is contemporary with the upper and mid-layers of Çatal East, Mentese, Erbaba, and Bademağacı, as well as middle phases at Ilipinar and Uğurlu (level V; see Atici et al. submitted). Finally, Ulucak IV is contemporary with Çatalhöyük West, Early Chalcolithic layers of Güvercinkayası and Köşk Höyük and the later Neolithic Level IV at Uğurlu.

Morphologically domestic cattle and pig were husbanded from the beginning of the settlement, together with sheep and goat. Based on the NISP (number of identified specimens) and relative bone weight (Fig. 2), while the emphasis on sheep and goat husbandry was mostly stable throughout time, the relative abundance of pig and deer remains (mostly *Dama*

Fig. 1 Location of Ulucak in Western Turkey in relation to other important contemporary sites

dama—fallow deer) does increase throughout the sequence, indicating increased exploitation (Table 2). Goats are always less numerous than sheep, despite the significantly large proportion of the caprine remains that could not be distinguished from one or the other. Sheep were probably more profitable to keep than goats in the well-watered plain within which Ulucak is situated. The significant increase in *Dama* might be related to a range shift or migration of the species, increased management of the species, or increased area of cultivation around the settlement (see Çakirlar 2016; Çakırlar and Atici 2017, and Sykes 2014 for further discussion on prehistoric *Dama* in western Turkey).

Overview of methods

Excavations at Ulucak are ongoing, but the material discussed in this study dates only from excavation years 1995–2012. The animal bones were collected by hand. The study was driven by results of the initial faunal analysis (Çakırlar 2012a, b), and additional unpublished data are reported here. Distinguishing between sheep and goat osteologically is a perennial challenge, as the existence of a number of manuals and articles attests (for example, Boessneck et al. 1964; Boessneck 1970; Davis 1987: 32–34; Helmer and Rocheteau 1994; Prummel and Frisch 1986; Payne 1985;

Table 1 Radiocarbon dates (n = 38) from Ulucak's Neolithic subphases (reproduced from 14sea.org)

Period	Levels and subphases	Max of calBC 1σ (from)	Min of calBC 1σ (to)	Number of published ^{14}C dates
Late Neolithic	IVb	5980	5710	2
	IVi	6030	5920	1
Middle Neolithic	Va	6220	5920	2
	Vb	6480	6080	7
	Vc	6430	6070	2
	Vd	6470	6050	5
	Ve	6650	6520	1
	Vf	6470	6080	6
Early Neolithic	VI	6770	6500	5
	VIa	7030	6460	5
	VIb	6770	6490	2

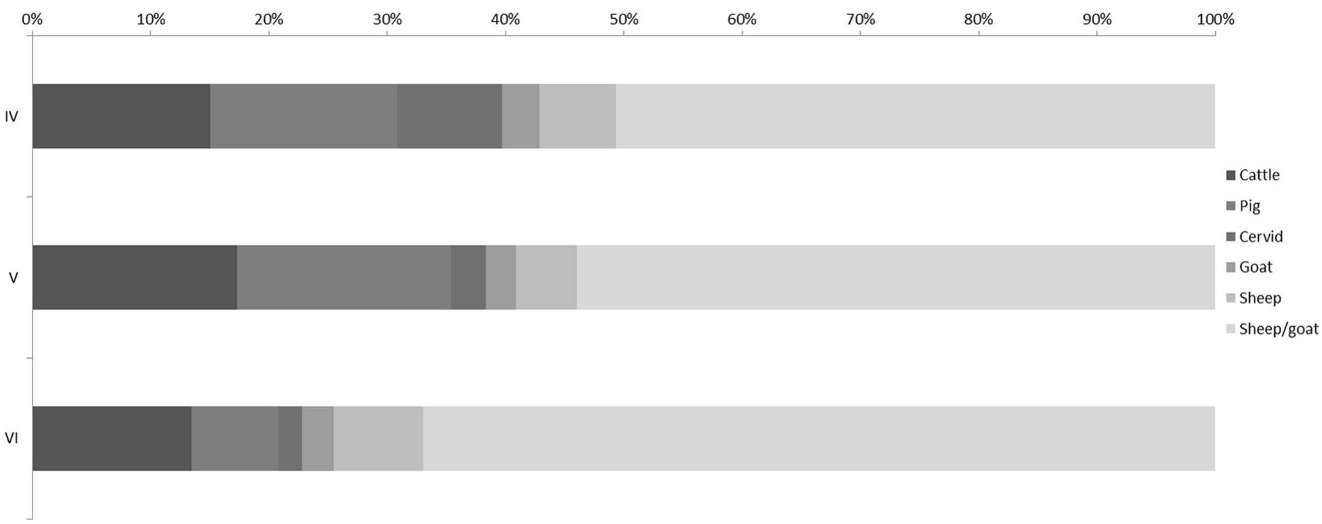

Fig. 2 Relative abundances based on NISP for cattle, sheep, goat, sheep/goat, pig, and deer (mainly *Dama*) per level at Neolithic Ulucak

Halstead et al. 2002; Zeder and Lapham 2010; Zeder and Pilaar 2010; Gillis et al. 2011). Yet, the distinction can be crucial for obtaining a more nuanced understanding of how these species may have been managed differently from one another and how that management may have diverged—or converged—through time, especially as compared to the use of other domestic taxa. This may include the intensification of use of cattle and pigs at the site in later periods, or a change from managing for meat versus secondary products such as wool and dairy. It could also be a response to local environmental change due to anthropogenic factors, territoriality, cultural choices, or changes in mobility patterns.

Ancient DNA analysis had long been considered as the only tool to clarify taxonomic affiliations in cases of unclear morphological traits, despite zooarchaeologists' best attempts at designing and testing standards for identification based on morphology. Traditional aDNA studies are often based on the amplification of mitochondrial DNA (mtDNA), a comparably well-preserved and maternally inherited extra-nuclear genome. On this basis, it is often not only possible to discriminate taxa, but, due to its high mutation rate, also between populations and/or sub-populations. However, post-mortem DNA degradation not only results in aDNA studies being a costly and time-consuming process, but also in high failure rates. This is particularly true for samples from the early Holocene and from warm climates, such as Neolithic Anatolia. Previous ancient mtDNA studies conducted on ancient animal bones from comparable contexts in Neolithic Bulgaria and Turkish Thrace as well as from Neolithic Ulucak (Scheu 2012; Geörg 2013; Scheu et al. 2015; Ottoni et al. 2013) gave rise to the hope that additional carefully pre-selected, visually well-preserved tooth samples would yield amplifiable amounts of ancient DNA to not only resolve questions of taxonomy and sex, but to also address detailed questions on the demographic history of the species.

Besides aDNA analysis, the discovery that the same collagen often used to date archeological bones contains enough differences in its peptide composition to separate sheep from goat (Buckley et al. 2009, 2010) offers an invaluable opportunity for the zooarchaeologist to better interpret faunal assemblages. The development of these protein (peptide mass) fingerprinting-based approaches has improved markedly over the last few years, primarily in relation to the throughput increasing into the analysis of thousands of samples (e.g., Brown et al. 2016; Buckley et al. 2016) and the widening of the taxonomic range beyond the domesticates (Buckley and

Table 2 NISP and weight totals for cattle, sheep, goat, sheep/goat, pig, and deer (mainly *Dama*) per level at Neolithic Ulucak. Based on updated dataset available on opencontext.org

Level	Cattle	Sheep	Sheep/Goat	Goat	Deer	Pig	Total NISP
IV	338	145	1141	71	199	357	2251
V	791	236	2467	117	134	829	4574
VI	527	295	2624	106	78	288	3918
Total							10,743
Level	Cattle	Sheep	Sheep/Goat	Goat	Deer	Pig	Total weight (grams)
IV	9313	1409	4725	854	2667	3887	22,854
V	17,822	3012	11,031	1516	1622	9356	44,360
VI	12,127	2451	8406	797	549	1729	26,060
Total							93,273

Kansa 2011) to wild terrestrial (Buckley and Collins 2011; van der Sluis et al. 2014; von Holstein et al. 2014), marine (Buckley et al. 2014), and micro-fauna (Buckley et al. 2016). This "fingerprinting" of unidentifiable bone fragments has allowed researchers to discover taxonomic affiliation of a limited number of species irrespective of the potentially high failure rates associated with aDNA analysis.

Measuring the ratio of the stable isotopes of oxygen and carbon present in the calcium carbonate ($CaCO_3$) of tooth enamel can allow for an indirect understanding of variability in diet, environment, and mobility. The oxygen isotope ratio ($\delta^{18}O$) in tooth enamel carbonate reflects the $\delta^{18}O$ of ingested meteoric water (Luz et al. 1984; Kohn 1996) which is related to ambient temperature, precipitation amount, relative humidity, and source moisture (e.g., Craig 1961; Dansgaard 1964). The carbon isotope ratio ($\delta^{13}C$) is indicative of carbon in the whole diet, derived from ingested plants. In herbivores, $\delta^{13}C$ of tooth enamel carbonate is generally 12–14‰ above that of diet (Lee-Thorp et al. 1989). Tooth enamel forms in increments that retain a discreet isotopic signature, recording a seasonal signal for a period usually spanning 1–2 years (Balasse et al. 2002). Although the rate of mineralization can vary depending on species physiology, individual genetics, nutrition, disease, and stress, the incremental nature of the growth is broadly comparable between different individuals so that inter- and intra-species comparisons can be made (Kohn and Dettman 2007; Hedges et al. 2005; but see also Reade et al. 2015). Because the completion of mineralization lags behind organic accretion, incremental samples will always represent a time-averaged signal (Balasse 2002; Balasse et al. 2002; Hoppe et al. 2004; Zazzo et al. 2005). The information available from incremental analysis of dental enamel is limited by the amount of time it takes one tooth to form and is dependent on species-specific variables. For example, in sheep, *Ovis aries*, an unworn lower second molar (M2) contains a 12-month record and the lower third molar (M3) forms over a period of 20–24 months in the second and third year of the animal's life (Balasse et al. 2002; Balasse et al. 2003). A crown height of 18 mm is therefore necessary to reflect at least a full year of growth (Balasse et al. 2012). Only M2s and M3s of fully adult individuals (i.e., those in which enamel growth has been completed; this is discernable by the stage of root formation) and exhibiting low degrees of wear on the occlusal (chewing) surface should be sampled, so that $\delta^{18}O$ and $\delta^{13}C$ values are representative of the maximum potential range of annual seasonal variability, i.e., 12 months. Whereas the stable isotope analysis of teeth can provide insight into seasonal variation early in life, bone collagen yields values that reflect an average diet over the last several years preceding death. Carbon values largely reflect vegetation, and the $\delta^{13}C$ of bone collagen is approximately 5‰ above diet, with approximately 7‰ carbonate-collagen spacing (Clementz et al. 2009; Krueger and Sullivan 1984). Nitrogen values are indicative of trophic level (Ambrose 1991), although they can also be influenced by aridity and sea spray.

Materials and sampling process

For details of the traditional zooarchaeological methods applied in faunal analysis, e.g., taxonomic identification, osteometry, and aging, see Çakırlar (2012a, b). So far, more than 33,000 vertebrate specimens from Neolithic Ulucak have been analyzed macroscopically. Out of this large assemblage, more than 7,000 specimens have been securely identified to sheep, goat, or sheep, or goat. Preservation, both due to human modification and post-depositional taphonomic factors, is poor (see Çakırlar 2012a). Only 145 mandibles with teeth were preserved well enough to enable scoring of tooth eruption and wear patterns following the scheme described in Grant (1982), including 49 mandibles with a single pre-molar or molar—a further factor limiting accurate reconstructions of preferred culling ages and a barrier to stable isotope analysis of teeth. However, larger teeth such as permanent molars are sampled through hand-collecting more frequently than milk pre-molars, as shown in previous studies (e.g., Payne 1972) and using tooth rows and/or mandible-teeth specimens to reconstruct culling patterns is considered to be the safer methodological approach.

Sample selection and metadata

Because of the destructive nature of sampling for biomolecular and biogeochemical analyses, a small number of specimens fulfilling specific parameters were chosen for these analyses following the initial faunal identification of 145 mandibles with teeth. These parameters include completeness of the tooth and eruption and wear stage. Samples for ZooMS and stable isotope analysis included 15 hemi-mandibles with teeth in varying stages of eruption and wear (Table 3). After samples were chosen, they underwent a blind test of identification criteria using Zeder and Pilaar (2010) by both Çakırlar and Pilaar Birch. Drilling of sequential samples of tooth enamel and bone sampling for stable isotope analysis was carried out first in order to ensure the suitability of the specimens and adequate sample size recovery. This preceded destructive sampling for aDNA and ZooMS since, although the latter sampling methods may use only the tooth root, the tooth may be damaged or destroyed if additional sample is required. Enamel subsamples were drilled with a 1 mm diamond-tipped drill bit at equal increments perpendicular to a single cusp of the M2 and/or M3. Samples were prepared and analyzed in the Department of Geology at Brown University (now Earth, Environmental, and Planetary Sciences). Fragments of mandibular bone (approximately 0.5 g) from 11 out of the 15 specimens were able to be analyzed for $\delta^{13}C$ and $\delta^{15}N$ of collagen in the Quaternary Isotope Paleoecology Laboratory

Table 3 Age scores for sampled mandibles with teeth (based on Grant 1982)

Zooarchaeological sample ID	d4	p4	m1	m2	m3
833	0	0	12	11	2
838	0	0	17	17	17
5525	0	0	0	12	12
7299	0	0	15	14	12
6062	0	12	12	12	8
7832	0	12	12	11	9
8621	0	0	13	12	12
8799	0	0	12	11	12
9457	0	0	0	12	10
11506	0	0	0	0	13
11526	0	0	13	12	9
12179	0	14	15	13	12
12475	0	14	19	17	13
13229	0	0	0	13	12
13268	0	0	0	11	9

at the Center for Applied Isotope Studies at the University of Georgia. These same 15 specimens were subsequently sampled for ZooMS at the University of Manchester. Finally, six visually well-preserved tooth roots (7299, 7832, 8621, 8799, 11506, and 12179) were assessed for survival of mitochondrial DNA in the dedicated ancient DNA laboratories of the Palaeogenetics Group at Johannes Gutenberg-University Mainz. Ancient DNA work was performed according to Scheu et al. 2015 with slight modifications described in the Supplemental Information. Specimen-level metadata are presented in Table 4.

Results

Faunal analysis, aDNA, and ZooMS

Out of all the remains, there were 145 specimens that included both mandibles and teeth, 67 with deciduous teeth, and 39 with the full permanent set (post Grant's a for the third molar), when they have the fourth premolar and the third molar. Tooth wear and eruption patterns indicate that changes take place in culling preferences throughout the sequence, suggesting shifts in production goals (Table 5). In the early half of the seventh millennium BC or early Neolithic (Ulucak VI), the majority of the sheep and goat individuals are culled between 6 months and 2 years, but a good 40% survive into post-prime ages, perhaps until 6 years of age (Fig. 3a). During the middle Neolithic, after 6500 BC (Ulucak V), there is an increase in juvenile cullings by up to 20% (age stage C and D), which can be interpreted as a turn to dairy products and more intensive herding focused on male cullings (Fig. 3b). In the late Neolithic Ulucak IV, where the sample size is smallest ($n = 20$), the proportion of post-prime age animals is the largest (Fig. 3c). This might indicate a greater focus on herd security or even a more pronounced emphasis on dairy and possibly wool production. Either way, the small sample of Ulucak IV does not indicate that the primary goal of keeping sheep and goat was meat production. One would expect accompanying shifts in management techniques, including changes in the mobility of the herds across the landscape, human-induced changes in the timing of birthing, and seasonality of culling.

None of the six ovicaprid teeth sampled for ancient DNA analysis from Ulucak yielded amplifiable amounts of mtDNA for sequencing. Previous comparable analyses from bones and teeth sampled at this site had a combined success rate of 32% for all four species analyzed (cattle, pigs, goat, and sheep; 16 out of 50 samples). Considering only ovicaprids, the success rate drops to 10% (2 out of 21 samples) (Geörg 2013; Scheu 2012). DNA preservation is largely correlated with climate and age, but also many other, site-dependent factors that are mostly unknown, for example, the pH value of the soil, the presence of enzyme inhibitors (e.g., humic acids), as well as the transpassibility (sponginess) of the bone element sampled (Bollongino and Vigne 2008; Bollongino et al. 2008). The Supplemental Information included for aDNA summarizes amplification success for the site of Ulucak from this and previous studies. The fact that no aDNA could be amplified from the six specimens of the present study is unfortunate, but given the results from previous work, it is not surprising.

Of the 15 sheep and goat mandibles submitted for ZooMS, five of these had been identified as "*Ovis/Capra*" because they lacked the features necessary for confident morphological/macroscopic identification (following Zeder and Pilaar 2010). Of the remaining 10 that were morphologically identifiable, eight were confirmed and two reassigned (c.f. Table 4). In total, three of the individuals were goats and 12 were sheep. These results make extrapolation of the isotopic data representative of species difficult. Based on ZooMS, the predicted ratio of goats to sheep in the assemblage would be 1:5; in contrast, based on osteological identification (total NISP), the ratio is generally closer to 2:5. This may lead to the over-representation of goats if using morphological identification alone, which was already shown by Zeder and Pilaar 2010; following this logic, out of 1000 indeterminate O/C remains, it would be inferred based on morphological distinctions that 400 (instead of closer to 200) bone fragments belonged to goats.

Stable isotope analysis

Out of the 15 specimens chosen for ZooMS and stable isotope analysis, one (8799) produced no usable stable

Table 4 Sample contexts and IDs

Zooarch sample ID	Level	Phase	Faunal ID sheep	Faunal ID goat	Faunal ID sheep/goat	ZooMS result	Tooth enamel carbonate ID	Tooth sampled	Collagen ID
833	Vb	Middle Neolithic			x	Sheep	–		LD20
838	Vb	Middle Neolithic			x	Sheep	–		LD23
5525	Ve	Middle Neolithic	x			Sheep	UT 16 + 17	M2 + M3	LD25
6062	IVd	Late Neolithic			x	Sheep	UT 9	M2	LD30
7299	VIab	Early Neolithic	x			Sheep	UT 4	M3	–
7832	IVc	Late Neolithic		x		Sheep	UT 11 + 12	M2 + M3	LD27
8621	IVb	Late Neolithic		x		Goat	UT 7 + 8	M2 + M3	–
8799	IVd	Late Neolithic	x			Sheep	–	–	–
9457	IVk	Late Neolithic	x			Sheep	UT 14 + 15	M2 + M3	LD29
11506	VIab	Early Neolithic	x			Sheep	UT 5	M3	LD22
11526	VIab	Early Neolithic	x			Sheep	UT 3 + 6	M2 + M3	LD24
12179	IVd	Middle Neolithic		x		Goat	UT 20 + 21	M2 + M3	LD26
12475	IVe	Middle Neolithic	x			Goat	UT 18 + 19	M2 + M3	LD28
13229	VI	Early Neolithic			x	Sheep	UT 1	M3	LD21
13268	VI	Early Neolithic			x	Sheep	UT 2	M3	–

isotope data. Twelve individuals were ideal for stable isotope analysis of tooth enamel ($n = 19$ teeth, with seven sample pairs, four single M3s, and a single M2) and the resulting isotopic data ($n = 106$) were normally distributed. Two individuals (833 and 838, *Ovis*) were not sampled for tooth enamel due to being too young and too worn, respectively. Eleven individuals retained enough mandibular bone material to be sampled for collagen analysis of $\delta^{13}C$ and $\delta^{15}N$. The primary diagenetic concern regarding the stable isotope analysis of C and N is the degree of preservation of bone collagen. All samples described here had an atomic C:N ratio falling between the accepted range of 2.9–3.6 (Ambrose 1990). This included nine individuals for which tooth enamel stable isotope data are available.

Table 5 Age stages (following Zeder 2006) of 145 *Ovis*/*Capra*, *Ovis*, and *Capra* mandibles in Ulucak Levels VI to IV

Age stage (Zeder 2006)	IV	V	VI
A	1	1	0
B	0	3	2
C	3	22	13
D	7	14	12
E	4	3	4
F	9	7	8
G	5	10	13
H	1	1	1
I	0	1	0
Subtotals	30	62	53
Total			145

Diet

Stable isotope analysis of bone collagen provides insight into average diet over several years; in the case of these animals, potentially the entire short lifespan. The mean values of $\delta^{13}C$ and $\delta^{15}N$ in the 11 individuals sampled for bone collagen stable isotope analysis are summarized in Table 6 and Fig. 4, below. Overall, the average $\delta^{13}C$ values reflect a diet comprised primarily of C3 vegetation (− 20.3‰) and a $\delta^{15}N$ signal typical of herbivores (6.0‰). There is little difference (< 1‰) between average $\delta^{13}C$ values for sheep and goat, suggesting a similar diet, with a larger range in values for sheep (~ 2‰) than in goat (~ 1‰), likely reflecting sample size differences. There is a slightly more notable difference in average $\delta^{15}N$ for the two species (5.8‰ for sheep and 7.1‰ for goat), with the same respective ranges in values as for $\delta^{13}C$. Though sample size is a limiting factor, there is no difference in bone collagen values between the two species. There are only minor differences through time periods at the site. In the early ($n = 3$) and middle Neolithic ($n = 5$), $\delta^{13}C$ values for sheep and goat average about − 20.5 ‰; this is very slightly more positive in the late Neolithic ($n = 3$), − 19.8 ‰. In all periods, the range of variability is the same (approximately 1.5‰). Nitrogen ratios through time are slightly more variable, particularly the larger range seen in values in the middle Neolithic (2.9‰) as compared to the early Neolithic (0.5‰) and late Neolithic (1.5‰); this range is not because of the inclusion of a juvenile in the sample (who may have elevated $\delta^{15}N$ values due to nursing) but rather the elevated $\delta^{15}N$ values in the two goats from this sample.

Because herbivore bioapatite $\delta^{13}C$ values will be more positive relative to the diet by as much as 12–14‰ (Lee-

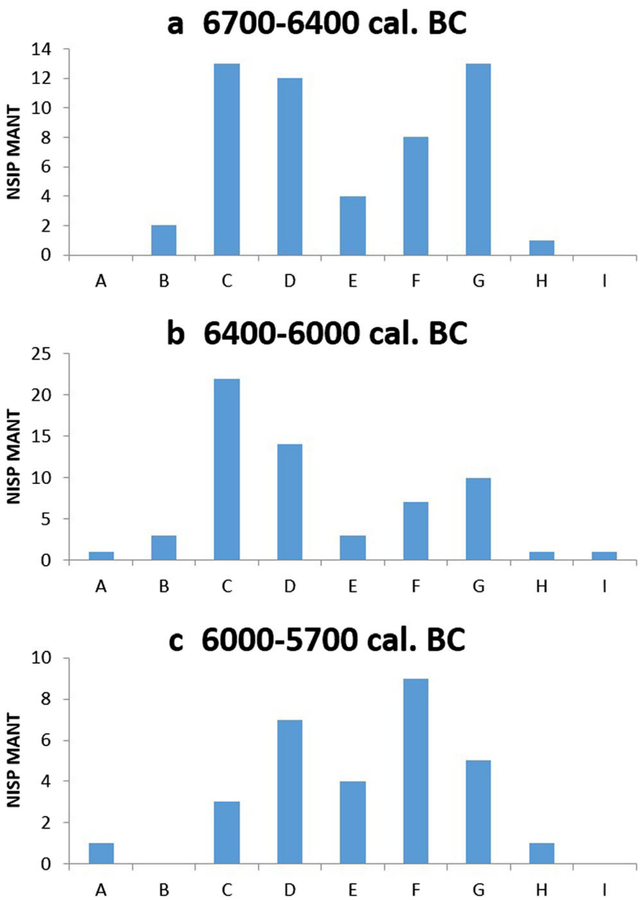

Fig. 3 **a** Age curve for the early Neolithic. **b** Age curve for the middle Neolithic. **c** Age curve for the late Neolithic

Thorp et al. 1989; Passey and Cerling 2002), the mean values of the majority of the teeth are expected to be offset to those of collagen by approximately 7‰. In this sample, the carbonate-collagen spacing is closer to an average of 8.5‰, with one individual exhibiting a difference of 11‰. The δ^{13}C values of between about 11 to 12 ‰ in the teeth support the interpretation of a predominantly C3 diet for all individuals (Fig. 4). Only one individual has a maximum value of around − 7 and a mean of about − 10‰ (11526), suggesting perhaps some limited consumption of C4 vegetation during the period of tooth formation that was aberrant from its lifetime average; this is the same individual with a 11‰ carbonate-collagen spacing.

Birth season

Because of the relationship of δ^{18}O values in tooth enamel carbonate to that of seasonal fluctuations in temperature and precipitation regimes, the intra-annual resolution of subsampling lends itself to an interpretation of birth season. Due to a lag in the accretion of enamel and therefore a delay in corresponding seasonality signal in δ^{18}O of teeth (c.f. Balasse et al. 2012; Henton et al. 2010), for spring lambs, δ^{18}O values progressing from the crown down to the neck of the M2 should be sequentially relatively lower δ^{18}O values, peaking at the lowest winter values before increasing to maximum recorded values, signifying summer, in a sinusoidal pattern. Most M2 teeth (UT 6, 9, 11, 16, 20) record values of around −1 to − 2 ‰ within the first 3 mm of the crown and exhibit a sinusoidal trend typical of a spring birth. Excluding individuals with more advanced M2 wear (UT 7 and 18), there is no apparent difference in birth seasons through time or by species, with the sole exception of UT 14 (a goat), which potentially had a late summer/early fall birth.

Ranges of variation

There was no relationship between the range of isotopic variation within a tooth and tooth crown height, nor was there a relationship between degree of isotopic variation and type of tooth (for example, M2 vs. M3). In addition, there was no relationship between within-tooth or within-individual isotopic variability and archeological site phase, although teeth from individuals in the middle Neolithic seemed to record the largest intra-tooth ranges δ^{18}O (> 5‰; Fig. 5) and moderate ranges of 2.5–4.2‰ in δ^{13}C. This could reflect different sources of water and graze/browse composition on a seasonal basis, perhaps in different locations. In contrast, there is less *inter*-tooth variation; this could suggest a tighter clustering of seasonal births or reflect the small sample size. Notably, there is also a larger range in the bone collagen results of δ^{15}N among individuals in the middle Neolithic than in the other two periods, which again may reflect a more varied management strategy or be a sample size effect. Overall, the isotopic variability exhibited in this sample is a function of individual life histories and is summarized in Table 7. Due to the small number of goat individuals ($n = 2$) compared to sheep, it is

Table 6 Summary of bone collagen stable isotope values from Ulucak specimens by species and phase

Species	Sample size	Mean ^{13}C	Range δ^{13}C	Mean δ^{15}N	Range δ^{15}N
Ovis	$n = 9$	− 20.2	2.1	5.8	2.2
Capra	$n = 2$	− 20.8	1.1	7.1	1.1
Phase	Sample size	Mean δ^{13}C	Range δ^{13}C	Mean δ^{15}N	Range δ^{15}N
Early Neolithic	$n = 3$	− 20.4	1.5	5.6	0.5
Middle Neolithic	$n = 5$	− 20.5	1.5	6.1	2.9
Late Neolithic	$n = 3$	− 19.8	1.3	6.2	1.5

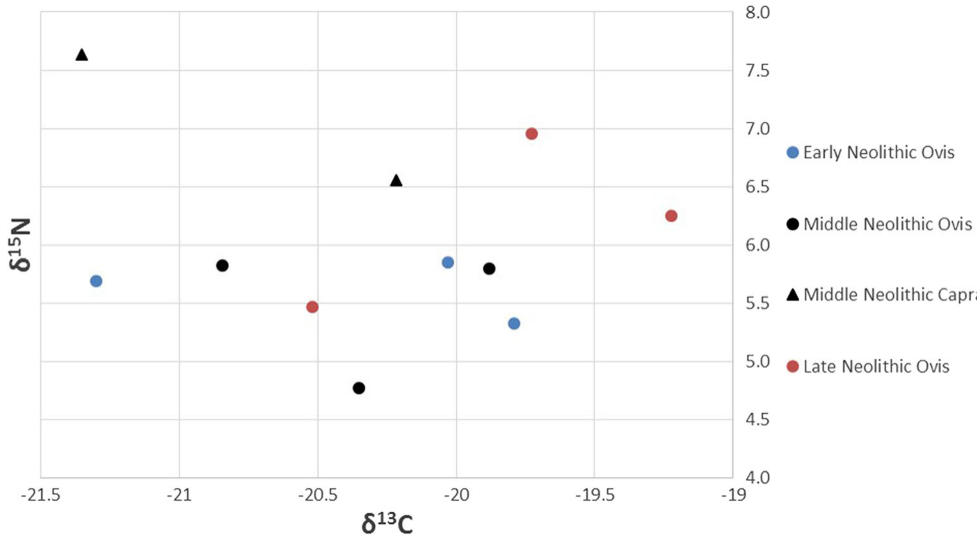

Fig. 4 Bone collagen stable isotope values from Ulucak specimens by species and phase

difficult to discuss interspecies variability. Based on the limited variability present in the isotopic data throughout time and between species, it would appear management strategies were conservative through time and that, based on their small numbers in the faunal assemblage in general (cf. Fig. 2), goats may very well have been managed along with the sheep.

Discussion

In this study, we wanted to further investigate how a shift in the faunal record might be reflective of changing management strategies of individual species, whether that meant treating sheep and goats differently, treating them the same, or altering degree of mobility or birthing intervals through time. Essential to achieving a species-specific, and potentially sex-specific, answer, we attempted aDNA analysis, which was unsuccessful. However, the field of ancient DNA has recently been revolutionized by the application of next-generation sequencing technologies and constantly optimized extraction and target enrichment methods that allow genome-wide analysis even of highly degraded ancient samples (see Metzker 2010; Orlando et al. 2015 for further reading). Careful pre-selection of particularly dense bones, such as petrous bones (Gamba et al. 2014) that contain particularly low amounts of contaminating and co-extracted environmental DNA, increases the chances of retrieving ancient DNA even from contexts such as Ulucak. The biggest challenge will be to uncover petrous bones together with the mandibles and other diagnostic postcranial elements, since archaeological animal bones are usually found as scattered remains further mixed over millennia. In this case, we were validated by turning to ZooMS as an alternative methodology. We would recommend using this approach in future studies where taxonomic affiliation and morphological identification is a concern; though not available for all species, the method is currently applicable to a number of taxa and growing and is viable even if there is no DNA preservation.

Small sample sizes and specimen bias are the nature of the archaeological record; in this case, the representation of goat vs. sheep in the isotopic sample was not ideal, leading us to consider ovicaprid management strategies as a whole in support of osteomorphological data as well as evaluate individual-level variation. While the archaeologist may anticipate "change" as the rule rather than the exception, in this case, there is relatively little differentiation in the diets, birth seasons, and mobility of sheep and goat through time in our sample. The zooarchaeological analysis suggests that the use of sheep and goat may have become more specialized through time and also less important as the relative proportions of cattle and pig increased.

Though there have been relatively few exhaustive stable isotope analyses of sheep and goat in the Neolithic in Turkey, it is notable that our isotopic results stand in stark contrast to those carried out on bone collagen from Çatalhöyük in Central Anatolia, which, in addition to having much elevated δ^{15}N values (9.4‰) and more positive δ^{13}C (− 18‰) indicative of a more arid environment and limited consumption of C4 plants, show a trend towards these higher values through time (Pearson et al. 2007). At Aşıklı Höyük, also located in Central Anatolia, there is no change through time, and there is no discernable difference in diet based on δ^{13}C (− 18.9‰) and δ^{15}N (8.3‰) from bone collagen in sheep and goat at either site (Pearson et al. 2007). The data for Ulucak align much more closely with unpublished data for δ^{13}C and δ^{15}N from bone collagen at Neolithic Yenikapi in the Marmara (Pilaar Birch and Cakırlar In preparation) and Uğurlu on the island of Gökçeada (Pilaar Birch et al. In preparation). Also at Neolithic Çatalhöyük, Henton et al. (2010) used oxygen isotopes in enamel primarily to discuss season of birth and ventured to suggest based on intra-tooth variability that despite a small sample size ($n = 4$) in the earlier

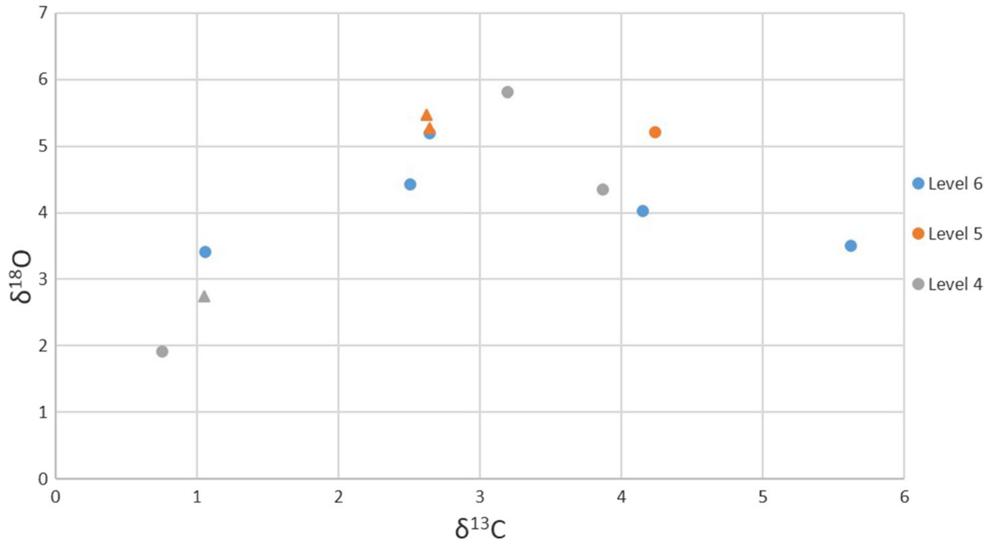

Fig. 5 Ranges of isotopic variation in individual animals. Triangles represent goats, and circles represent sheep

phase of occupation it seemed most sheep spent the year close to the settlement, whereas two individuals from later phases may have traveled as far as 30 km away and 600 m in elevation in the summer. This does not seem to be the case at Neolithic Ulucak.

Conclusions

This is the first time osteological, aDNA, ZooMS, and stable isotope analyses were applied in combination in a Neolithic assemblage in Anatolia and Southwest Asia. We view this as a formative study demonstrating the great potential of the utility of combining multiple methodologies in order to ask how we might better address nuance in the archaeological record and go beyond conventional discussions of livestock use. Although our attempts to extract sufficient aDNA from the bone samples were not successful with the methods we applied, the results of the ZooMS analysis confirmed the conclusions of the Zeder and Pilaar 2010 study, by showing in this case study that the comparative osteomorphology criteria commonly applied to distinguish sheep and goat from their mandibular teeth are biased towards goat, causing over-representation of this species in the zooarchaeological record. Perfecting species identification is particularly important for sites like Ulucak that fall outside of the traditional borders of "core" domestication areas but are investigated for the timing and nature of the appearance of different domesticates and how they mixed with local wild populations (Arbuckle et al. 2014; Ottoni et al. 2013). Although we usually talk about the arrival or appearance of sheep and goat at the same

Table 7 Summary stable isotope data from tooth enamel carbonate and bone collagen by individual

Tooth enamel carbonate ID	O_{Min}	O_{Max}	O_{Mean}	O_{StdDev}	O_{Range}	C_{Min}	C_{Max}	C_{Mean}	C_{StdDev}	C_{Range}	Collagen ID	$\delta^{15}N$	$\delta^{13}C$
UT 1	−3.8	1.4	−1.1	2.1	5.2	−13.0	−10.4	−12.0	1.1	2.6	LD21	5.85	−20.03
UT 2	−3.2	1.2	−1.5	1.6	4.4	−13.2	−10.7	−12.0	1.0	2.5	–		
UT 4	−1.9	1.5	−0.1	1.5	3.4	−12.8	−11.8	−12.2	0.4	1.1	–		
UT 5	−3.8	0.3	−1.7	1.4	4.0	−13.6	−9.5	−11.4	1.5	4.1	LD22	5.32	−19.79
UT 3 + 6	−3.6	0.1	−2.0	1.3	3.5	−12.6	−6.9	−10.3	2.1	5.6	LD24	5.69	−21.3
UT 16 + 17	−3.4	1.8	−0.5	1.7	5.2	−13.3	−9.1	−11.4	1.6	4.2	LD25	5.8	−19.88
UT 18 + 19	−4.9	0.6	−1.7	1.7	5.5	−14.9	−12.2	−13.1	0.8	2.6	LD28	7.64	−21.35
UT 20 + 21	−1.7	3.5	0.3	1.7	5.3	−13.5	−10.8	−12.1	0.7	2.7	LD26	6.55	−20.22
–											LD20	4.77	−20.35
–											LD23	5.83	−20.84
UT 7 + 8	−2.2	0.6	−0.6	1.0	2.7	−12.5	−11.4	−11.8	0.3	1.1	–		
UT 9	−3.3	−1.4	−2.4	0.9	1.9	−11.8	−11.0	−11.4	0.3	0.8	LD30	6.25	−19.22
UT 11 + 12	−2.8	1.6	−1.1	1.2	4.3	−13.8	−9.9	−11.8	1.2	3.9	LD27	6.96	−19.73
UT 14 + 15	−4.8	1.0	−1.1	1.6	5.8	−13.5	−10.3	−12.0	1.2	3.2	LD29	5.47	−20.52

time in western Anatolia and the Aegean region at large, and in several other regions of secondary Neolithization, corroborating this through aDNA or ZooMS might in fact be compulsory. The ZooMS results imply that goats may be less abundant than observed using morphological criteria. The stable isotope results from tooth enamel carbonate imply that Ulucak VI and V may not differ significantly in terms of the way sheep and goat were managed, despite alterations in the culling strategies. The stable isotope results from bone collagen also imply continuity in environmental parameters and diets of small ruminants, contrasting to results from Çatalhöyük and Aşıklı Höyük, and adding to the increasing differences between Central Anatolian and Western Anatolian Neolithic (Arbuckle et al. 2014; Çilingiroglu and Çakirlar 2013; Çilingiroğlu 2017). While sample sizes are necessarily constrained by available material, funding, and preservation, we advocate for as large a sample as possible when subjecting remains to multiple biomolecular and biogeochemical analyses, and the integration of methods during the project planning stage rather than an ad hoc basis in order to sample in the most efficient order. Building on the current primary focus on the absence, presence, and origins of domestic animals in regions of Neolithization, and understanding conservatism and change in early farming cultures, will require a detailed and accurate record of animal exploitation strategies in these areas towards which this study is a first step for the eastern Aegean Neolithic. The authors hope that it may serve as a model for current and future research in the region and beyond.

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